scanning from m/z 50 to m/z 335 in 1.5 sec. MS/MS spectra of unknowns were compared to those of standard compounds acquired under the same instrumental conditions for confirmation of identity.

Primer design

Primer design for each allele was based upon reference to the *B. pseudomallei* K96243 genome project (http://www.sanger.ac.uk/). Genomic DNA for PCR amplification was purified using the MasterPure™ DNA purification kit according to the manufacturer's instructions (Epicentre Technologies, Madison, WI). Internal gene fragments were PCR amplified with the primer pairs listed in Table 2 using the following conditions: one cycle at 94°C for 5 min, 30 cycles at 94°C for 30 sec, 56°C for 30 sec, 72°C for 30 sec, followed by a final 7 min extension at 72°C. For confirming site-specific integration, the extension time was increased to 4 min. All PCR reactions were performed with the Epicentre FailSafe kit using buffer "J" (Epicentre Technologies). Reactions were analyzed on a 0.8% agarose gel containing ethidium bromide (43) and subcloned into pCR2.1-TOPO (Invitrogen, Carlsbad, CA). Ligations were transformed into One Shot® chemically competent *E.coli* (Invitrogen) and screened by standard methods (43).

Mutant construction and confirmation:

Disruption cassettes were made by digesting pCR2.1-TOPO containing internal gene amplicons for each of the eight quorum loci with *EcoR*1 (New England Biolabs, Beverly, MA) for 1 hr at 37°C. Digestions were heat inactivated and subcloned into the suicide vector pGSV3 (McClean et al., 1997, supra) using the Epicentre Fast-Link DNA ligation kit (Epicentre Technologies). Ligations were chemically

transformed as described above and screened on LB plates containing 10 μg/ml of gentamycin (Sigma). Random colonies (five from each transformation) were inoculated into 2 ml of LB broth containing 10 μg/ml of gentamycin and incubated at 37°C for 16-18 hr with agitation. Plasmid DNA was purified using the Wizard Plus Miniprep kit (Promega, Madison, WI), digested as described above, and analyzed on a 0.8% agarose gel with ethidium bromide. Clones containing inserts were electrically transformed into *E. coli* SM10 and mobilized into *B. thailandensis* DW503~~, Ricky, is this supposed to be B. mallei, or B. pseudomallei~~(Simon et al., 1989, supra). Transconjugants were selected on LB plates containing 10 μg/ml of gentamycin and 15 μg/ml of polymyxin (Sigma). Genomic DNA from transconjugants, three mutants from each mating experiment, was purified using methods described above. Site-specific integration, indicated by a 3.0 Kb increase in amplicon size corresponding to the suicide vector, was confirmed using PCR methods previously described for target gene amplification incorporating an extension time of 4 min.

Whole body Aersol exposures:

Approximately 48 hr prior to challenge 3 ml cultures were individually inoculated with wild-type *B. mallei* and each quorum mutant and incubated for 24 hr at 37°C. A 1 ml aliquot from the 3 ml overnight cultures was used to inoculate 25 ml of LBG. Cultures were incubated at 37°C for 18 hr, optical densities (OD_{660}) measured, and 10 ml (approximately 10^9 colony forming units/ml) was delivered to groups of 10 mice via nebulization using methods described by Jeddeloh et. al.(2002, supra). Chamber concentration was determined by CFU enumeration from air samples collected within the exposure compartment and the relative inhaled